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MEASUREMENT OF THE ENVIRONMENTAL EFFECTS ASSOCIATED WITH FORESTRY USE OF ROUNDUP R

by

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ABSTRACT

Herbicides are becoming an increasingly popular tool in forestry for the control of competitive vegetation. Roundup ^{R}, which contains the active ingredient glyphosate, is now the herbicide used almost exclusively for this purpose. The data base on the environmental effects of glyphosate is much smaller than that of the chlorophenoxy herbicides which were previously popular. Accordingly, a number of studies were undertaken to determine the environmental effects of Roundup ^{R} and, in some instances, to compare these effects with those documented for 2,4-D and 2,4,5-T.

The deposit of ground and aerially applied glyphosate on flat plates was measured using rhodamine dye as a tracer. Applications were at normal and twice normal operational dosage rates. Deposit was highly variable. However, ground applications provided the highest rate of deposit inside the target area with no measureable off-site deposits. Aerial sprays resulted in measureable deposit up to 95 metres off target.

The persistence of glyphosate on foliage and litter from aerially sprayed plots was also measured. Foliage selected for study were from red maple (Acer rubrum), balsam fir (Abies balsamea), raspberry (Rubus spp.) and grasses (Posceae and Cyperaceae). Highest concentrations of glyphosate were detected the day after spraying in raspberry and grasses (13 and 12 ug/g dry weight respectively). Red maple, forest litter and balsam fir had smaller concentrations of 5.1, 2.6 and 0.09 ug/g dry weight, respectively, one day after spray.

Residues in all substrates, with the exception of forest litter, declined rapidly up to the last day of sampling, 58 days post spray. Forest litter residues demonstrated an overall decline, however, the rate was less rapid than in foliage.

The rate of decomposition of litter, both in the field and in the laboratory, after treatment with herbicide was also explored. Substrates studied were forest floor material (litter, duff and humus), and fresh foliage from red maple or white spruce (Picea glauca). Samples of substrate were either dosed in the laboratory or placed in field plots which were subsequently sprayed with herbicide. The amount of substrate decomposition decreased with increasing dosages of 2,4-D, 2,4,5-T, a 50:50 mixture of 2,4-D and 2,4,5-T, and glyphosate. These effects, however, were only significant at concentrations greater than 1000 ug/g. Such concentrations are considerably in excess of those associated with operational spraying.

The final study determined stream residue concentrations following direct oversprays at operational dosage rates of glyphosate and evaluated whether such concentrations affect stream periphyton abundance. Artificial substrates were placed in a stream above and below the target area, sampled at various times after spray and analyzed for chlorophyll a, phaeophytin, biomass and ATP content. Stream residues were highly variable immediately after spray with the highest concentration (39 ug/L) being measured 30 hours post spray. While periphyton abundance was variable, no changes which could be attributed to pesticide effects were noted for the study period which concluded 37 days after spraying.

RÉSUMÉ

Les herbicides deviennent un outil de plus en plus populaire en foresterie pour le contrôle d'espèces végétaes compétitrices. Roundup [R], lequel contient l'ingrédient actif glyphosate, est maintenant l'herbicide utilisé presque exclusivement à cette fin. Le littérature sur les effets environnementaux du glyphosate est de beaucoup inférieure à celle sur les herbicides chlorophenoxy qui étaient duparavant plus populaire. Consequemment, un certain nombre d'études furent entreprises afin de déterminer les effets sur l'environnement causés par l'utilisation du Roundup [R] en Nouvelle-Ecosse et dans certain cas, de comparer ces effets avec ceux documentés pour 2,4-D et 2,4,5-T.

La première étude avait pour but de mesurer les dépots de glyphosate sur des plaques lorsqu'appliqué de façon dériènne ou terrestre en utilisant comme traceur un colorant au rhodamine. Des taux de dosage normaux ainsi que deux fois ces taux furent utilisés lors des applications. Les dépots étaient hautement variables, cependant les applications terrestres ont démontrés les plus haut taux de dépots à l'intérieur de la zone cible avec aucun dépot mesurable à l'extrieur de la cible. Les applications dériènnes ont montrés des dépots mesurables jusqu'à 95 mètres à l'extérieur de la zone cible.

La persistance du glyphosate sur le feuillage et sur les feuilles tombées sur le sol lorsqu'appliqué de façon aérienne à aussi été mesurée par technique GC. Les feuillages analysés provenaient d'rables rouges (Acer rubrum), sapins (Abies balsamea), framboisiers (Rubus spp) et d'herbes (Posceae et Cyperaceae). Les plus hauts taux de glyphosate furent détectés le jour après l'application dans les framboisiers et les herbes (respectivement 13 et 12 ug/g poids sec). L'érable rouge, les feuilles mortes et le sapin avaient respectivement des concentrations de 5.1, 2.6 et 0.09 ug/g poids sec une journée après l'arosage.

Les résidus dans tous les substrats, avec l'exception des feuilles mortes, ont diminué rapidement jusqu'au dernier jour d'chantillonage 58 jours après l'arosage. Les résidus de feuilles mortes ont démontré dans l'ensemble un déclin, le taux était cependant moins rapide que dans le feuillage.

Le taux de décomposition des feuilles mortes, sur place et dans le laboratoire, après traitement avec herbicide fut aussi exploré. Des substrats furent collectés de matériaux organiques déposés sur le sol, d'érables rouges ou d'epinettes blanches (Picea glauca). Des échantillons de substrat furent soit dosé dans le laboratoire ou laissés sur place à des endroits qui furent arrosés avec des herbicides par la suite. La décomposition de substrat decroissait avec l'augementation des dosages de 2,4-D, 2,4,5-T, de glyphosate et d'un mélange 50-50 de 2,4-D et de 2,4,5-T. Ces effets étaient seulement significatifs (p moins de 0.01) lorsque les concentrations etaient superieures à 1000 ug/g. Ainsi, les herbicides étudiés se sont montrés suffisamment toxiques pour réduire le taux de décomposition des feuilles tombées sur le sol seulement à des concentrations bien supérieures à celles que l'on s'attend suite à un arosage.

L'étude finale avait pour but de déterminer les concentrations de résidus dans un cours d'eau suite a un arosage normal de glyphosate et de vérifier si ces concentrations affectaient l'abondance de periphyton dans le cours d'eau. Des substrats artificiels furent placés dans un cours d'eau en amont et en aval de la zone cible et furent échantillonés à plusieurs reprises après l'arosage et analysés pour chlorophylle a, phaecophtine, biomasse et teneur en ATP. Les résidus dans le cours d'eau étaient très variable immédiatement après l'arosage avec la plus haute concentration (30 ug/g) obtenue 30 heures après l'application. L'abondance de periphytons était variable, cependant jusqu'à 37 heures après l'arosage, aucun changement attribuable à l'herbicide n'a été noté.

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1.0 INTRODUCTION

Glyphosate [N-(phosphonomethyl) glycine] has been known to have herbicidal activities since the early 1970s (Baird et al., 1971). Like the chlorophenoxy herbicides, glyphosate is translocated (Coupland, 1985) and is a broad-spectrum, post emergence herbicide which is active on virtually all plants (Franz, 1985). Conifers, however, are relatively tolerant to glyphosate and can be released from competition with broadleafed plants by the administration of appropriate doses of the herbicide (a treatment commonly referred to as "conifer release"). Available information indicates that glyphosate inhibits the shikimic acid pathway of plants, resulting in a reduction of protein synthesis and prevention of secondary compound formation (Cole, 1985). Other effects of the chemical, which may be a factor in its herbicidal activity, include its inhibitory effect on chorophyll synthesis and indole-3-acetic acid (IAA) production, as well as an ability to promote ammonium accumulation (Cole, 1985).

The margin of safety for glyphosate in animals is acceptable, since they do not have a shikimic acid pathway (Cole, 1985). The observed acute toxicity to mammals (Sullivan and Sullivan, 1979; EPS, 1982; Ghassemi et al., 1982), birds (Ghassemi et al., 1982), aquatic invertebrates and fish (Folmar et al., 1979; Hildebrand et al., 1980; Singh and Yadav, 1978) is low. The published data base for glyphosate, however, is surprisingly small for a chemical which is used extensively in both agriculture and forestry. In particular, the effects of glyphosate on soil litter decomposition, microbial activity, and aquatic primary production have not been adequately addressed. In addition, there has been no work in Atlantic Canada on the chemical's persistence in foliage, even though its use for silvicultural purposes in the region is increasing.

Accordingly, a number of studies were undertaken following aerial conifer release spraying, to determine: (a) deposition of herbicide spray; (b) residues in target foliage and the forest floor; (c) effects on litter decomposition rate; and (d) changes in stream periphyton.

2.0 SPRAY DEPOSIT

The deposit of pesticide inside and outside the target area is highly variable in any spray program. Ideally, the application should achieve a uniform deposit within the target area, with minimal drift. By maximizing on-site deposition, off-target contamination is minimized and a greater proportion of the spray reaches the intended target (Norris, 1967).

In general, aerial spraying gives a more even spatial distribution than ground-spraying, since tractor-mounted equipment must contend with slope of terrain, slash, clumps of stump sprouts, and other obstacles (Kieley and Ernst, 1984). Aerial spraying, however, generally presents a greater risk of drift due to the increased distance spray particles must travel to reach their target, and due to generally smaller droplet size (Barry and Ciesia, 1981).

The available literature on pesticide spray distribution during forestry operations is generally more concerned with drift than with spatial distribution in the spray block. In addition, most studies have dealt with forest insecticide spray programs or agricultural situations. Both of these are inherently different from conifer release programs due to such factors as canopy height, vegetation cover, and terrain.

This study examined ground and aerial conifer release herbicide applications to determine spray deposition within and immediately outside the target area. The two sites sprayed by air were treated with glyphosate and the two sites which were ground-sprayed were treated with 2,4,5-T. Since deposition was measured using rhodamine dye as a tracer, and since the formulation characteristics of the glyphosate and 2,4,5-T sprays were very similar valid comparisons could be made between ground and aerial applications.

2.1 Materials and Methods

To monitor spray distribution, 165 cm^2 plastic petri plates were placed at regular intervals to form a grid within each spray area. Between 56 and 91 sample points were located in each spray block. At each sampling point, one collector was set above the vegetation canopy on a 1.3 m stake, and another positioned at ground level. Additional plates were placed at ground level at approximately 15 m intervals up to 150 m outside the spray block. Prior to spraying, Rhodamine WT dye was added to the herbicide spray solution (in an 1800 L mixing tank) at ca. 0.5% to serve as a tracer. Dye was mixed in the spray solution in an amount proportional to the amount of active ingredient.

Immediately following the spray application, the plates were collected. Each plate was rinsed with ethanol into glass vials, and the solutions were stored for up to two days in the dark at 2°C until analysis. A spectrophotometer (Bausch and Lomb Spectronic 88) was used to analyze samples at a wavelength of 545 um. Concentrations of rhodamine were determined by comparison with dye standards.

The four sites (Table 1) were sprayed by Nova Scotia Department of Lands and Forests as part of their conifer release program. Two sites (Riverside and Cope's Hill) were ground-sprayed by a tractor-mounted boom-jet cluster-nozzle, with a spray height of 3 m. The other two sites were aerially treated with a helicopter using a boom and nozzle (Tee Jet D6 cones), flying at 115 km/h, 17 m above the ground. Manufacturers specifications for this type of delivery indicate a droplet size of 300-400 um. Weather data were recorded at the beginning of each spray operation, and subsequently at 15-minute intervals.

All four sites had a spray block treated at the standard silvicultural application rate (1x), and three of the four sites also had double application (2X) spray blocks. Table 1 summarizes spray

TABLE 1 CHARACTERISTICS OF THE SPRAY BLOCKS

Site	Location	Treatment	Plot Area (ha)	Application Rate a.i. (kg/ha)	Spray Volume (L/ha)	Mode of Application	Time Sprayed	Herbicide
Riverside	50°10'N;	1X	4.1	3.37	835	ground	Sept./83	2,4,5-T
	47°00'W	2X	5.9	6.74	835	ground	Sept./83	2,4,5-T
Cope's Hill	49°80'N;	1X	7.2	3.37	835	ground	Sept./83	2,4,5-T
	49°05'W							
Little River	49°81'N;	1X	11.9	1.14	55	helicopter	Aug./84	glyphosate
Lake	49°40'W	2X	3.8	2.28	N/A	helicopter	Aug./84	glyphosate
Antrim	49°78'N;	1X	9.6	1.67	56.3	helicopter	Aug./84	glyphosate
	46°80'W	2X	11.0	3.34	N/A	helicopter	Aug./84	glyphosate

N/A = Not available.

rates and the location and size of various spray plots, plus other pertinent information. The weather parameters recorded at the time of spraying are summarized in Table 2. Application rates were calculated by dividing the total emitted volume by the total area treated.

2.2 Results

Results of the deposition study are presented in Table 3. For ground-sprays, mean values of measured deposition ranged from 22% to 86% of applied spray. The two 1X ground applications gave the highest deposit (Riverside 1X = 86%; Cope's Hill = 70%), whereas only 22% was measured for the Riverside 2X application. Aerial sprays generally had lower target deposits, ranging from 18% to 59% of applied spray. Aerial spraying at the 1X rate yielded 59% at Antrim and 23% at Little River Lake. Deposits were greatest for sprays made early in the day, when windspeeds were predictably low (i.e. Riverside 1X and Antrim 1X). For other spray events, deposit was not clearly related to windspeed data. However, weather data were collected periodically, and thus do not accurately reflect the influence of wind gusts on deposition.

The actual emitted spray rates were different from those intended for the silvicultural treatments (Table 4), ranging from 34% to 135% of the intended rate. This inefficiency, coupled with actual deposition of emitted spray, meant that only 11% to 46% of the intended application rate was achieved, in terms of spray deposited on the collectors.

Dye could not be detected on any collection plate situated 10 m outside of the spray blocks for ground-spray applications. In contrast, plates located outside of the aerial spray plots showed measureable deposits. Fifteen metres outside the Antrim 1X spray block, 9 collection plates had an average ($\pm$ S.D.) dye deposition of 6.0 $\pm$ 2.3 ug/plate (equivalent to 2.8% of average deposition on the spray plot). Outside the Antrim 2X plot, deposition at 10 m and 20 m

TABLE 2 WEATHER CONDITIONS AT THE TIME OF HERBICIDE SPRAYING*

Site	Treatment	Spray Date	Time of Day	Temperature		Windspeed		Relative Humidity	
				Min.	Max.	Min.	Max.	Min.	Max.
				(°C)		(km/h)		(%)	
Riverside	1X	Sept. 11/83	0845-1015	12	16	0	1	86	90
	2X	Sept. 11/83	1015-1230	16	23	0	13**	72	86
Cope's Hill	1X	Sept. 14/83	1540-1935	15	22	0	11**	64	82
Antrim	1X	Aug. 29/84	1025-1035	22	22	3	7	91	96
	2X	Aug. 29/84	1040-1110	22	25	3	6	84	96
Little River Lake	1X	Aug. 29/84	1325-1400	30	31	5	5	66	68
	2X	Aug. 29/84	1315-1325	30	31	5	5	66	68

* Measurements made 1.5 m above ground.

** Spraying delayed or interrupted due to wind gusts.

TABLE 3 DYE DEPOSITION FOR THE VARIOUS SITES AND TREATMENTS. MEAN ± S.D. FOR DYE DEPOSITION; MEAN ± 2 S.E. FOR PERCENT DEPOSITED OF EMITTED DYE

Site	Treatment	Application Rate	Number of Collectors	Dye Deposition (ug/plate)	Range of Deposition (ug/plate)	Percent[a] Deposited (%)	Canopy Penetration[b] (%)
Riverside (ground)	above canopy	1X	91	912 ± 1069	0 - 5021	86 ± 19	72
	ground			659 ± 679			
	above canopy	2X	84	382 ± 402	0 - 1671	22 ± 18	80
	ground			308 ± 425			
Cope's Hill (ground)	above canopy	1X	56	644 ± 658	0 - 2649	70 ± 15	41
	ground			261 ± 360			
Antrim (aerial)	above canopy	1X	58	214 ± 170	0 - 689	59 ± 15	46
	ground			98 ± 108			
	above canopy	2X	59	153 ± 79	5 - 322	18 ± 15	95
	ground			145 ± 96			
Little River Lake (aerial)	above canopy	1X	57	102 ± 73	22 - 395	23 ± 14	86
	ground			88 ± 58			
	above canopy	2X	59	266 ± 163	2 - 773	21 ± 21	63
	ground			167 ± 135			

[a] Percent Deposited = $\frac{\text{Dye Deposit in Collectors}}{\text{Actual Emitted Dose}} \times 100$

[b] Canopy Penetration = $\frac{\text{Dye Deposit in Collectors Below Canopy}}{\text{Dye Deposit in Collectors Above Canopy}} \times 100$

TABLE 4 INTENDED, EMITTED, AND DELIVERED SPRAY RATES FOR THE VARIOUS SITES AND TREATMENTS. DELIVERED RATES ARE IN MEAN ± 2 S.E.

Site	Treatment	Intended Spray Rate (kg a.i./ha)	Emitted Spray Rate[a] (kg a.i./ha)	Deposited Spray[b] (kg a.i./ha)	Emitted (% of intended)	Deposited (% of intended)	Deposited (% of emitted)
Riverside	1X	3.37	1.14	0.95 ± 0.21	34	28	86
	2X	6.74	3.36	0.75 ± 0.61	50	11	22
Cope's Hill	1X	3.37	2.22	1.55 ± 0.30	66	46	70
Antrim	1X	1.67	1.23	0.71 ± 0.18	74	44	59
	2X	3.34	2.61	0.47 ± 0.39	78	14	18
Little River Lake	1X	1.14	1.21	0.28 ± 0.17	106	25	23
	2X	2.28	3.08	0.65 ± 0.65	135	29	21

[a] Emitted Spray Rate = $\frac{\text{Amount of Dye Ingredient Sprayed}}{\text{Area Actually Treated}}$

[b] Deposited Spray = Deposit on Plates Above Canopy Expressed as kg a.i./ha

averaged 2.7 ± 3.2 ug/plate (n=13) and 1.3 ± 0.6 ug/plate (n=6), respectively (1.7% and 0.8% of mean deposition on the spray plot). At Little River Lake, off-site deposition showed a sporadic decline along a 150 m transect running perpendicular to a spray-plot boundary (Table 5).

Deposition frequency histograms, which plot the grouped deposit on plates against the total number of plates falling within that category, are presented in Figure 1. The data demonstrate that on-site deposition from aerial spraying was more homogeneously distributed than deposition from ground-spraying (Figure 2). Ground-sprays had coefficients of variation (i.e. S.D./mean) greater than 100% (range of 117%-192%; Table 3); the coefficients were smaller (range of 61%-79%) for aerial applications, indicating less spatial heterogeneity in the latter.

Canopy penetration was calculated as the difference in deposition between ground collectors and above-canopy collectors. Results were highly variable, presumably due largely to heterogeneity in vegetation cover. Among all sites, mean values of canopy penetration ranged from 41% to 95%.

2.3 Discussion

In spray deposit studies, on-site deposition rarely accounts for all of the sprayed material (Maybank et al., 1974). According to Akesson and Alwady, 1969, cited in Ware et al. (1971), deposition can vary from 30%-95% of aerially applied agricultural spray, depending on weather conditions. Other factors such as canopy structure and distribution, mode of application, spray altitude, or spray droplet size also influence degree of deposition to the spray block (Warren, 1976).

Previous studies of spray deposition have found widely varying results. Norris (1967) cited unpublished data of Newton,

TABLE 5 OFF-SITE DEPOSITION ALONG A TRANSECT RUNNING PERPENDICULAR TO THE BOUNDARY OF THE 1X SPRAY PLOT AT LITTLE RIVER LAKE. APPLICATION WAS BY HELICOPTER

DISTANCE FROM PLOT EDGE (m)	DYE DEPOSITION (ug/plate)
Spray plot average	184
25	1.8
35	0.7
50	0.0
65	1.2
80	2.3
95	0.4
110	0.0
125	0.0
140	0.0
150	0.0

Figure 1

Frequency Histogram of Spray Deposition at the Antrim and Little River Lake Plots (Rotary Wing Application)

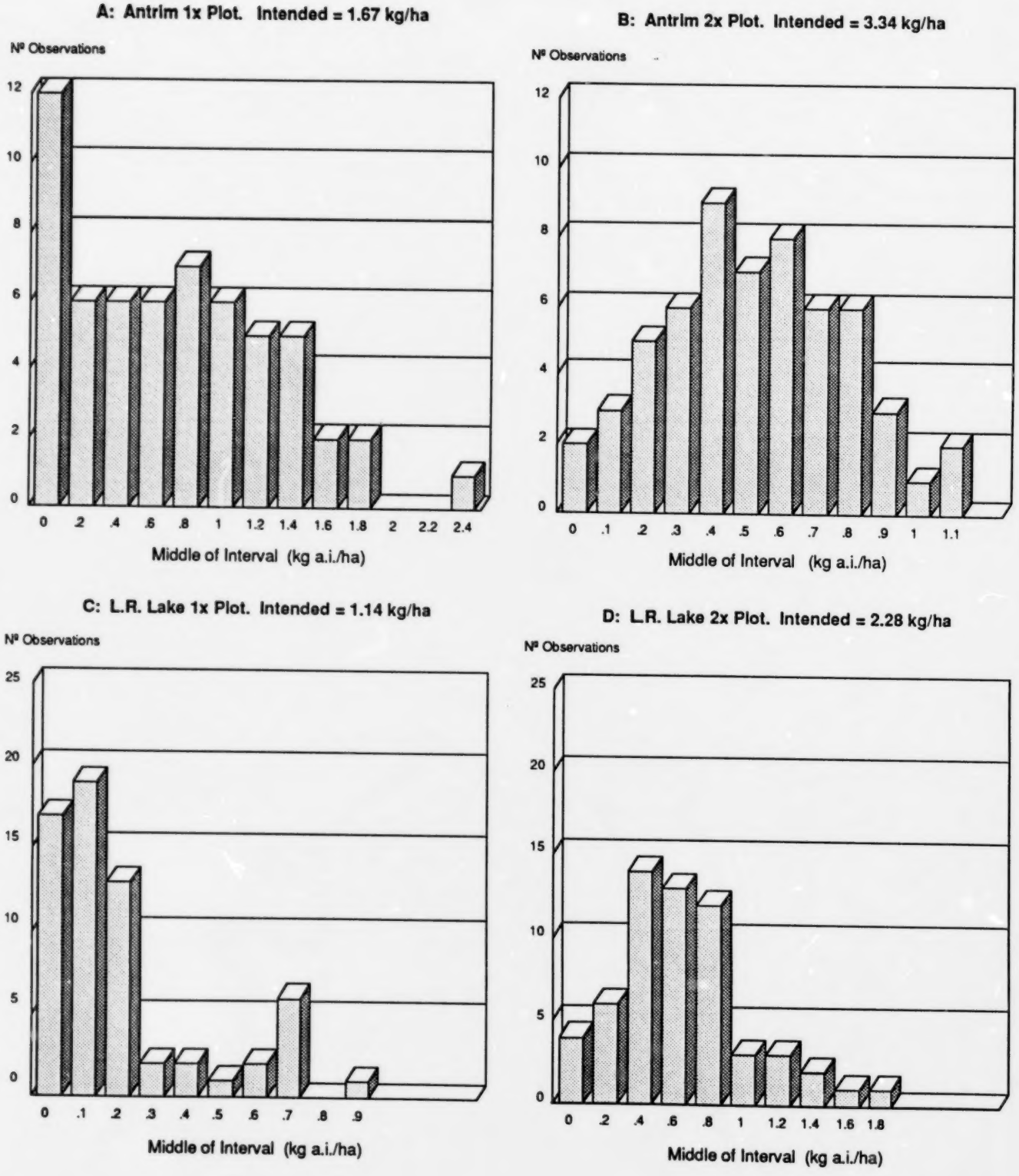
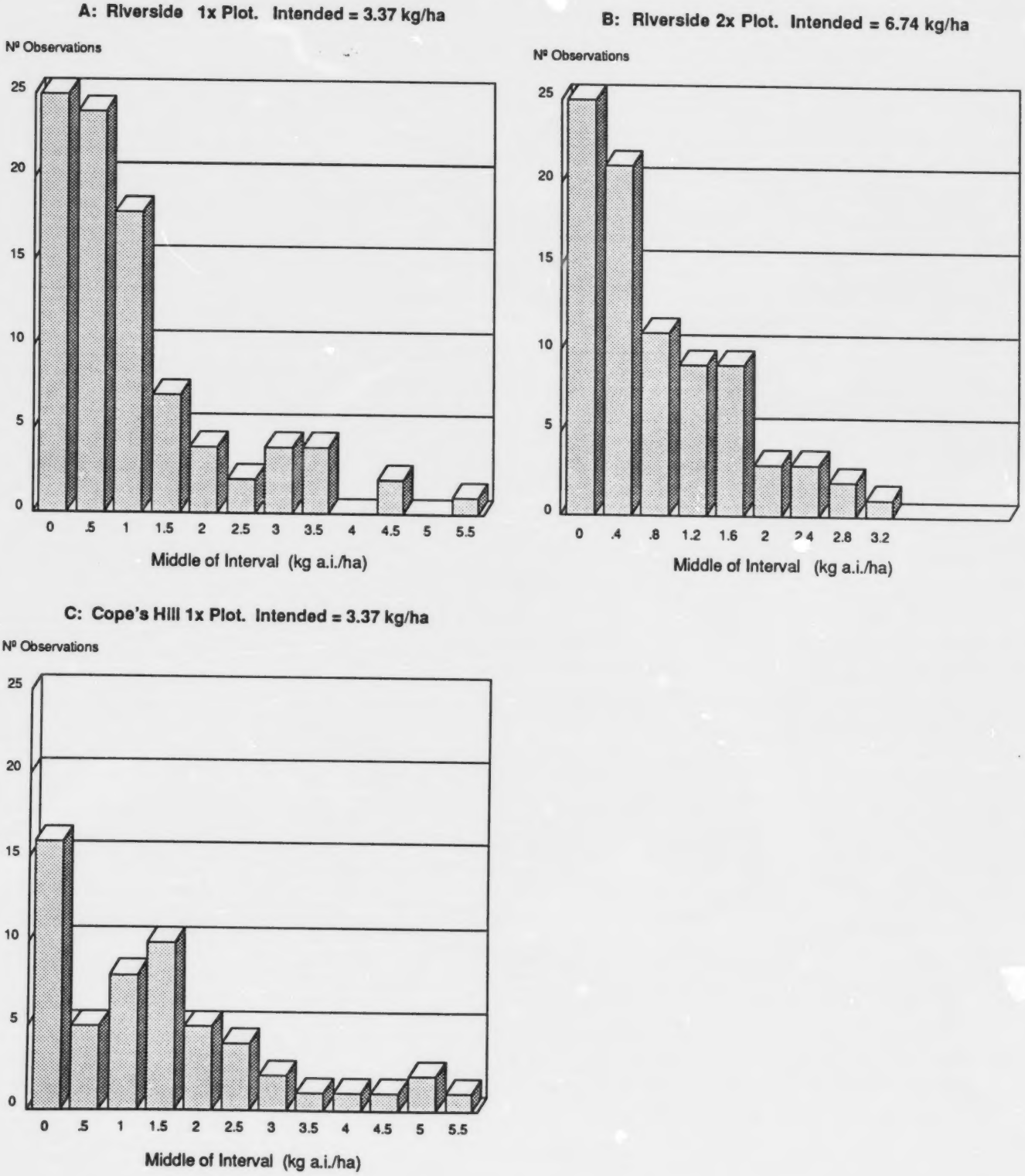


Figure 2

Frequency Histogram of Spray Deposition at the Riverside and Cope's Hill Plots (Ground Application)



Norris and Zavitkovski for spray deposition during forestry herbicide spraying. In a trial where a Christmas tree plantation was sprayed by fixed-wing aircraft, on-site deposition accounted for 75% of emitted spray volume. In another fixed-wing application for conifer release, however, deposition was only 25%-40% of the emitted volume. By way of comparison, a deposition of 60% was reported for a helicopter application.

In three (i.e. Riverside 1X 86%, Cope's Hill 1X 70%, and Antrim 1X 59%) of seven situations evaluated in this study, a relatively high deposition rate, compared with emitted dose was observed. In the remaining trials deposition was less than 25%.

Deposition rate is affected by several factors. For example, spray plots with occasional standing trees or tree islands force aircraft to fly higher, leading to greater opportunity for drift through greater fall heights. In this study, emitted spray rates were frequently lower than desired due to inadequacies in calibration (Table 4). These inefficiencies, coupled with off-target movement of the spray cloud, resulted in an actual deposit (expressed as a percentage of intended spray rate) of only 11%-46% for the seven spray sites. Kieley and Ernst (1984) also reported low herbicide deposit for three spray sites in western Nova Scotia (21% and 5.1% of intended spray rate for two aerial applications, and 1.7% for a ground application).

The relatively poorer deposition for aerial applications compared to ground sprays may be partially attributable to the higher release altitude required in the former. The longer droplet hang times which allow for evaporation of diluent and an consequential increase in the relative proportion of small droplets. Smaller droplets are much more susceptible to drifting and aerodynamic drag which would decrease deposition on the flat collection surfaces (Ekblad, 1978). Casual observation of collection plates indicated

that much larger droplets were deposited by the ground spray equipment.

Some researchers have concluded that the difference between total emitted spray, and spray deposited on the target is not an adequate measurement of off-target drift (Maybank, 1969; Maybank and Yoshida, 1973). Possible sources of error include: (1) inaccurate spray area calculations; (2) dish edge shielding (dependent on droplet trajectory); and (3) systematic errors associated with a grid of collection plates (Maybank and Yoshida, 1973). Grover et al. (1977) suggested that random placement of collectors could reduce variability of deposition measurements in agricultural situations. However, mobility and difficulty in re-locating collectors in clearcuts can be problems with a random sampling design.

Swath patterns have been measured in agriculture (e.g. Grover et al., 1977) and for certain situations in forestry (e.g. Norris et al, 1978; Lavy et al., 1980). This study examined deposit patterns throughout the spray block. A greater awareness of these patterns could facilitate understanding of the heterogeneity of plant community responses following herbicide application. This could be important in ensuring an adequate release of conifers from competition. The results of this investigation suggest aerial application results in a more uniform deposit than does ground application.

3.0 PERSISTENCE OF GLYPHOSATE IN FOLIAGE AND LITTER

The degree to which a herbicide persists in the environment has important implications in determining long term environmental impacts. Persistence of 2,4,5-T in the forest environment has been extensively studied in the United States (Norris, 1981). However, persistence of glyphosate has not been as thoroughly researched in the forestry context. Information from agricultural studies indicates that the longevity of glyphosate residues depends on the characteristics of the environmental compartment. For example, glyphosate in a clay loam and muck soil is rapidly deactivated, with half lives in such soil estimated at 28 days (Rueppel et al., 1977; Sprankle et al., 1975a, 1975b; Torstensson and Stark, 1979). In contrast, Lund-Hoie (1975) demonstrated that plant metabolism was disrupted up to two years after application of glyphosate to various species of plants.

The purpose of this study was to investigate glyphosate persistence in some common plant species and in forest floor substrates from clearcuts sprayed for conifer release in Nova Scotia.

3.1 Study Sites for Glyphosate Persistence

Two study sites were selected. The Antrim site was a mixedwood clearcut, planted in 1982 with black (Picea mariana) and red spruce (P. rubens). Competing vegetation was primarily red maple (Acer rubrum), raspberry (Rubus spp.) and grasses (Poaceae, Cyperaceae). Little River Lake was initially an old-field, white spruce, balsam fir stand that, after clearcutting, was planted with black spruce in 1982. Grasses and various herbaceous dicots were the major competitors, along with alders (Alnus spp.) and raspberry. Both sites were sprayed with Roundup^{R} by helicopter on August 29, 1984. Rates of application were 1.67 kg a.i./ha for Antrim and 1.14 kg a.i./ha for Little River Lake.

3.2 Methods

Foliage of red maple, current year growth of balsam fir (Abies balsamea), and surface forest floor litter (mixed conifer and deciduous) were collected over an 8-week period at the Antrim site. Raspberry and grasses were collected at Little River Lake. Foliage was obtained from the outermost branches of individual plants (at approximately 2 metre heights) in locations where there was no over-topping vegetation. At each sampling, sufficient foliage was collected to fill a 500 mL acetone and hexane rinsed glass container, and then deep-frozen until analysis, approximtely one year after collection. Homogenous samples were created by freeze-drying and grinding prior to analysis.

Analysis of glyphosate followed the method outlined in the FDA Pesticide Analytical Manual (Anon, 1982), with one important difference. Following the advice of J. Cowell (Monsanto, pers. comm.), samples were macerated in a 1:3 chloroform-deionized water mixture, and then centrifuged. The water phase was decanted, and the procedure repeated after adding more deionized water to the chloroform extract. After vacuum filtration, the extract was diluted with deionized water to 1800 mL and passed through an anion exchange column. The rest of the procedure followed the FDA method. No attempt was made to separate the glyphosate from its major metabolite (aminomethyl phosphonic acid) during the cation exchange step. Subsequently, diazomethane was used to derivitize the glyphosate residues.

Analysis was carried out on a Tracor 222 gas chromatograph fitted with a nitrogen-phosphorus flame photometric detector. Gas flow rates (mL/min) were as follows: air-27, oxygen-57, nitrogen-100, hydrogen-200. Temperatures were: column-150°, detector-180°, injection port-225°. A standards calibrater curve obtained from Monsanto Inc. was used to determine sample values. A standard-sample-standard sequence of injection was employed as

recommended in the FDA method. The calculated level of detection for this analysis was 0.01 ug/g.

3.3 Results and Discussion

Analytical results are presented in Table 6. Percent recovery was evaluated using two 100 ug/g spikes in litter substrates run in duplicate. One spike produced 58% and 60% recoveries, while the second spike resulted in 68% and 65% recoveries (mean recovery was 63%). Residue concentrations reported are not adjusted for recovery rates.

Red maple, conifer and forest floor samples were collected after spraying at the Antrim site (Table 6). Red maple had 5.2 ug/g of glyphosate on the day of spraying, but decreased to 0.45 ug/g one week later. Residues after 2, 4, 6 and 8 weeks were 0.47, 0.06, 0.03 and 0.12 ug/g respectively. Fluctuations in concentration were minor considering the non-uniformity of spray deposition (see Section 2). Residues in the conifer samples were only detectable for the day of spraying and one week later (0.09 and 0.04 ug/g, respectively). Since the conifers were overtopped by competing vegetation, it was expected that they would not have concentrations as high as the more exposed species. In the forest floor, glyphosate residues were 2.6 ug/g initially, and dropped to 1.1 ug/g one week later. Values further declined over time with the exception of the 8-week sample which had a 2.0 ug/g content.

Raspberry and graminoid (grass) samples were collected at Little River Lake. On the day of spraying, 13 ug/g and 12 ug/g were detected in raspberry and graminoids, respectively. These declined to 0.35 ug/g and 1.4 ug/g one week later. As with the red maple samples from Antrim, glyphosate concentrations in the raspberry foliage at 6 and 8 weeks post-spray were slightly in excess of values determined in the 4-week sample.

TABLE 6 GLYPHOSATE RESIDUES* (ug/g dry wt.) IN PLANT FOLIAGE AND FOREST FLOOR SAMPLES AFTER A SILVICULTURAL RELEASE SPRAYING

	DAYS AFTER SPRAYING					
	1	8	16	29	42	58
Antrim						
Red Maple	5.2	0.45	0.47** 0.12**	0.06	0.03	0.12
Balsam Fir	0.09	0.04	L0.01	L0.01	L0.01	L0.01
forest floor	2.6	1.1	0.89	0.50 0.40**	0.35	2.0
Little River Lake						
Raspberry	13	0.35	0.14** 0.14**	0.21	-	0.25
Grasses	12	1.4	0.76	-	-	0.46

* Detection limit = 0.1 ug/g.

** Replicate samples.

L = less than

All foliage samples showed large declines in concentrations in the first week after spraying. This may be partially due to movement within the plants, since glyphosate is readily translocated (Sprankle et al., 1975a). As the major metabolite (aminomethyl phosphonic acid) was not measured, it is impossible to determine if the decrease in residue concentrations were due to metabolism of glyphosate within the plant. In addition, rain occurred two days after spraying and this may have caused some wash-off. In the first post-spray week, rainfall occurred on 5 of 7 days at Little River Lake (total = 28 mm) and on 4 of 7 days at Antrim (total = 14 mm).

The residue concentrations in vegetation in this study are lower than those reported in a similar study by Newton et al. (1984), however, Newton et al., 1984 monitored applications of 3.3 kg a.i./ha. The trends observed in both studies are similar, with glyphosate residues declining rapidly after spraying.

4.0 EFFECTS OF HERBICIDE TREATMENT ON LITTER DECOMPOSITION

Many aspects of nutrient cycling are strongly related to processing of detrital materials (Crossley, 1970; Gottschalk and Shure, 1979). Decomposer organisms break up and oxidize plant and animal detritus, and in the process recycle inorganic nutrients. They ultimately process 90% of net primary production in forest systems (Mason, 1977; Gottschalk and Shure, 1979). Thus, anything which affects rates of litter decomposition in forests can be expected to have important ecological implications.

Little attention has been paid to the possible effects of herbicide use on litter decomposition in forests. Potentially these effects could be direct, by toxicity of herbicide residues to decomposing organisms, or indirect as a result of herbicide-caused changes in vegetation, with secondary effects on litter decomposition by changes in microclimate.

Herbicides can reach the forest floor either directly during silvicultural application, or indirectly due to the washing action of rain or as residues in or on leaves that dehisce from treated plants (Upchurch, 1966; Norris, 1981). The presence of these herbicide residues could potentially disrupt litter decomposition (Gottschalk and Shure, 1979). Of particular concern are potential effects in the forest floor, as this is the major zone of decomposition activity (Mason, 1977).

The purposes of the present study were to determine: (a) whether the presence of glyphosate could affect decomposition rates in litter bioassays (for purposes of comparison, data are also presented on the effect of 2,4-D and 2,4,5-T on decomposition rates); and (b) whether litter decomposition in the field is affected by herbicide caused changes in vegetation and microclimate.

4.1 Materials and Methods

4.1.1 Laboratory Study

Herbicides used in this experiment were 2,4-D; 2,4,5-T; a 50:50 mixture of these (DT), and glyphosate. All were formulated as emulsifiable concentrates. They were diluted with distilled water and applied to each substrate. The amount of water used was standardized as the quantity required to thoroughly wet the substrate without over saturation, and was identical in all treatments at 1.5 parts water: 1 part substrate. The wetted substrate was well-mixed to ensure an even distribution of water and herbicide. Herbicide application rates were 0 (control), 1, 10, 100, 1000, 5000, and 10,000 ppm of active ingredient, dry weight. The concentrations undoubtedly declined during the course of the experiment, due to dissipatation, however, this effect was not measured.

4.1.1.1 Substrates

Three substrates were used: foliage of two tree species, and forest floor (composed of litter, duff, and humus). Foliage substrates were leaves of either red maple or white spruce (Picea glauca), collected in June, 1984. Both of these tree species are important on many sites where herbicides are sprayed for forestry purposes in Nova Scotia. Forest floor material was collected from a mesic mixed-hardwood stand in central Nova Scotia.

Each substrate was air-dried, passed through a grinder, sieved at 0.6 mm, and then mixed to produce a homogenous material. Two hundred grams of forest floor substrate was placed in each of 130 4.5 L polyethylene wide mouth containers. This allowed 5 replicates for each herbicide treatment and a control group of 10. One hundred grams of spruce foliage substrate was placed in each of 130 4.5 L containers and the same amount of red maple foliage was placed in 110 4.5 L containers. This allowed 5 replicates per herbicide treatment

and 10 controls for spruce foliage, and 4 replicates per herbicide treatment and 10 controls for the maple foliage. The distilled water-herbicide mixture was added, and the contents well-mixed.

Containers were then covered with plastic film (Saran Wrap) to reduce moisture loss, and placed in a greenhouse for incubation (112 days for forest floor substrate, 123 days for spruce foliage, and 121 days for maple foliage). Mean temperature during the incubations was 26°C. Evaporation losses were monitored by weighing the container plus contents to 0.1 grams, and moisture lost was replaced tri-weekly.

At the end of the incubation period samples were oven-dried at 105°C to constant weight. Initial dry weights of air-dried substrates were calculated from air-dry:oven-dry weight ratios determined using subsamples. Difference in dry weight before and after the incubation period was considered loss due to decomposition.

Analysis of variance was conducted on each data set. Tukey's test of Least Significant Difference was used to determine which herbicide concentrations had statistically significant effects on decomposition. A two-way analysis of variance was applied to both the foliage and forest floor data sets to investigate differences between herbicides.

4.1.2 Field Study

Four grams of air-dry foliage of red maple were placed in each of 72 litterbags. These were constructed of fibreglass window screening, and were envelope-shaped with dimensions of 25 cm x 13 cm. Initial dry weights were calculated using ratios of air-dry : oven-dry (105°C) weights determined on subsamples of foliage.

Litterbags were placed at one of the treatment sites

(Riverside) prior to spraying. The three field plots were all approximately 7 ha in size, and herbicide treatments were: (a) unsprayed control; (b) a plot sprayed at a conventional silvicultural application rate of 3.4 kg 2,4,5-T/ha; and (c) a double-spray plot treated at 6.7 kg 2,4,5-T/ha. Each treatment-plot had 12 permanent 5 m x 5 m quadrats for vegetation studies, located in a grid. Two litterbags were placed at opposite corners of each quadrat, for a total of 24 per treatment. Litterbags were exposed for the first post-spray year (Sept. 1983 to Sept. 1984), after which they were collected, and the contents oven-dried and weighed. Percent weight loss was calculated from the difference between initial and final dry weights, and herbicide treatments were compared by analysis of variance.

4.2 Results

4.2.1 Laboratory Study

Data for the red maple leaf decomposition are summarized in Table 7. Decomposition of controls averaged 34% of initial foliage weight. While there were general decreases in weight loss with increasing amounts of all four herbicides, these differences were only statistically significant ($p < 0.05$) at the highest concentrations (5,000 and 10,000 ppm) of 2,4-D, 2,4,5-T, and DT treated replicates, and at 10,000 ppm for glyphosate.

Data for spruce needle decomposition are summarized in Table 7. Weight loss of controls was similar to the maple foliage substrate, and averaged 32%. While there was a general decrease in weight loss with increasing amounts of all four herbicides, these effects were only significant at concentrations of 5,000 and 10,000 ppm. The only exceptions to this were 2,4,5-T at 1000 ppm, and DT at 1 ppm. 2,4-D had much greater effects ($p < 0.01$) on decomposition at 5000 and 10,000 ppm, than did the other three herbicides.

TABLE 7 EFFECTS OF HERBICIDES ON DECOMPOSITION OF SEVERAL LITTER SUBSTRATES. DATA ARE IN PERCENT WEIGHT LOSS, MEAN (S.D.).

Substrate	Concentration	2,4-D		2,4,5-T		DT		Glyphosate	
	(ppm)	% Wt Loss	n	% Wt Loss	n	% Wt Loss	n	% Wt Loss	n
a) Acer rubrum	0	34.3 (1.6)	10	34.3 (1.6)	10	34.3 (1.6)	10	34.3 (1.6)	10
foliage	1	35.7 (1.6)	4	36.4 (1.6)	4	33.8 (0.7)	4	33.4 (2.9)	4
substrate	10	33.5 (0.9)	4	33.8 (0.3)	4	33.2 (1.1)	4	33.1 (1.3)	4
	100	32.7 (0.4)	4	33.6 (0.6)	4	34.3 (1.0)	4	32.8 (0.4)	4
	1,000	32.9 (0.4)	4	31.2 (0.9)	4	33.6 (0.4)	4	31.2 (0.4)	4
	5,000	28.5 (0.6)**	4	27.8 (0.8)**	4	29.5 (0.5)*	4	31.8 (0.6)	4
	10,000	25.1 (0.5)**	4	23.2 (.16)**	4	26.3 (0.4)**	4	28.8 (0.8)**	4
b) Picea glauca	0	31.6 (1.6)	10	31.6 (1.6)	10	31.6 (1.6)	10	31.6 (1.6)	10
foliage	1	29.1 (2.7)	5	32.3 (1.7)	4	27.8 (2.4)	5	29.8 (0.9)	4
substrate	10	28.8 (1.3)	4	31.7 (2.9)	4	28.0 (1.3)	4	30.0 (0.5)	4
	100	29.8 (2.4)	5	28.7 (2.6)	5	28.7 (1.9)	5	28.5 (2.6)	5
	1,000	30.3 (0.9)	5	27.8 (0.9)*	5	28.9 (0.7)	4	28.5 (1.1)	5
	5,000	18.3 (0.8)**	5	25.6 (2.2)**	5	24.6 (3.2)**	5	27.4 (2.3)*	5
	10,000	14.3 (2.0)**	4	26.7 (3.1)**	5	23.4 (3.5)**	5	25.7 (1.2)**	5
c) forest floor	0	6.3 (0.5)	10	6.3 (0.5)	10	6.3 (0.5)	10	6.3 (0.5)	10
substrate	1	5.2 (1.2)	5	6.2 (0.7)	5	8.2 (0.8)*	5	6.3 (0.5)	5
	10	6.1 (0.3)	5	6.2 (0.7)	5	6.7 (0.4)	5	6.3 (0.5)	5
	100	11.1 (0.8)**	5	6.3 (0.6)	5	7.1 (1.9)	5	5.7 (0.8)	5
	1,000	11.2 (0.5)**	5	5.2 (0.4)	5	5.4 (0.4)	5	5.5 (1.1)	5
	5,000	8.6 (1.0)**	5	3.8 (0.5)**	5	4.1 (0.8)**	5	4.9 (2.1)	5
	10,000	7.2 (0.5)**	5	2.9 (1.1)**	5	2.7 (0.2)**	5	3.5 (1.3)**	5

* = significantly different from control at p L 0.05.
** = significantly different from control at p L 0.01.

Data for decomposition of the forest floor substrate are summarized in Table 7. Weight loss was much less than for either foliage substrate, with controls averaging 6%. This is to be expected, since forest floor organic matter is relatively complex and resistant to decomposition, in comparison to freshly fallen leaves. Examination of the data for 2,4,5-T, DT, and glyphosate revealed a decrease in weight loss with increasing amounts of herbicide. These differences were only statistically significant at concentrations of 5,000 and 10,000 ppm for 2,4,5-T and DT, and 10,000 ppm for glyphosate. A different trend was observed for 2,4-D, where decomposition increased with addition of herbicide to the substrate. These 2,4-D effects were significant at concentrations of 100 ppm.

4.2.2 Field Study

Dry weight loss of red maple leaf litter exposed for the first post-spray year was (mean $\pm$ S.D., n = 24) 51.6% $\pm$ 2.1% on the unsprayed plot, 50.6% $\pm$ 2.7% on the 3.4 kg 2,4,5-T/ha plot, and 51.8% $\pm$ 3.0% on the 6.7 kg 2,4,5-T/ha plot. These differences were not significantly different ($p \text{ L } 0.10$).

4.3 Discussion

In order for herbicides to exert a toxic effect on microorganisms involved with litter decomposition, concentrations must occur in the forest floor or litter that exceed the threshold for measureable effect. In general, the toxic thresholds which we observed in our laboratory study were much higher than concentrations of residues which are generally seen in the field. At the same time, it must be recognized that the community of microorganisms present on fresh foliage surfaces are not the same as those in forest floor litter. The effects of glyphosate on each microorganism community could be quite different.

For operational use in forestry, expected initial field concentrations of 2,4-D and 2,4,5-T generally range from 5-20 ppm in the forest floor and rarely exceed 100 ppm (Kearing and Kaufman, 1969; Norris, 1981). Expected initial concentration of glyphosate ranges from 5-10 ppm in the forest floor (Rueppel et al., 1977; Newton et al., 1984). Such initial residue levels correspond to field application rates ranging from 2.3-9.0 kg a.i. herbicide/hectare for phenoxy herbicides (N.R.C.C., 1978) and 1.7-5 kg a.i. herbicide/ hectare for glyphosate (Ghassemi, 1982).

Herbicides in the forest floor are subject to dissipative processes, including volatilization, adsorption, leaching, and uptake by plants (Bovey and Young, 1981). There is considerable evidence to show that 2,4-D is rapidly decomposed in the forest floor (Upchurch, 1966; Norris, 1981; Bovey and Young, 1981). Foster and McKenchen (1973) reported that half-lives of agriculturally applied 2,4-D ranged from 14-41 days depending on soil type and Plumb et al. (1977) reported a soil half-life of 19 days. Norris (1981) reported that half-lives of phenoxy herbicides are dependent upon initial concentration in the soil. All of these studies agree that persistence of 2,4-D in soils is short, with ca. 90% degraded within 40 days. Similar studies with 2,4,5-T indicate a half-life about two times greater than for 2,4-D (Bovey and Young, 1981; Norris, 1981). This is due, in part, to the greater difficulty that microorganisms have in breaking it down (Upchurch, 1966). Nevertheless, 2,4,5-T degraded at a moderate rate, with 90% gone after the first 90 days (Norris, 1981; Bovey and Young, 1981).

Degradation of glyphosate is a microbial process (Sprankle et al., 1975a; Torstensson and Aamisepp, 1977). Breakdown due to either photodecomposition or chemical degradation is minimal (Rueppel et al., 1977). Past studies have shown a wide variation in fate of glyphosate in forest soils. Rueppel et al. (1977) found that 90% of glyphosate dissipated from forest soils within 12 weeks. Allan and Klein (1984) found glyphosate only in the top 6 cm of the forest floor after

spraying, where it disappeared rapidly. In laboratory studies Sprankle et al. (1975a) found an initial rapid disappearance of glyphosate from soil and then, 7 days after application, a slower but steady rate of loss. Under operational conditions, Newton et al. (1984) reported a half-life of 10-24 days in the litter zone of an Oregon forest. However, Torstensson and Stark (1979) found that an average of 20% of glyphosate remained in six Finnish soils 8 months after spraying. This variation in persistence may be due to level of microbial activity in soil, and to herbicide availability in the soil environment (Moshier and Penner, 1978). Therefore, factors which influence microbial activity, such as pH, moisture, temperature, organic substrate availability, and nutrient levels can be expected to affect rate of glyphosate degradation (Sprankle et al., 1975a; Muller et al., 1981).

Effects of herbicides on detrital organisms are not usually great. Fletcher (1980) and Bollen (1961) found that phenoxy herbicides did not adversely affect total microbe numbers or activities at recommended application rates up to 6 kg/ha. Only at extremely high concentrations are there significant disruptions of microbial population dynamics or decomposition processes (Atlas, 1978). Furthermore, not all microbes respond in the same way. Depending on the herbicide, some microbes can withstand quite high concentrations, while others are severely affected (Bovey and Young, 1981). However, it is generally recognized that 2,4,5-T is more toxic to greater numbers of microbes than other phenoxy herbicides (Upchurch, 1966).

The effect of glyphosate on specific groups of microorganisms has been discussed by several authors. While there have been reported cases of some very low concentrations of glyphosate (less than 5 ppm) inhibiting activity of certain microbes (Jaworski, 1972), other studies have found that higher concentrations had no measurable effect. Torstensson and Stark (1979) and Muller et al. (1981) studied

the effect on nitrogen cycling of glyphosate at concentrations of 100-160 ppm in different soils, with no inhibitory effects observed.

The above literature concurs with results of our leaf litter and forest floor substrate decomposition studies. While amount of decomposition decreased with increasing amounts of all four herbicides, there were only significant effects at concentrations greater than 1000 ppm. Thus, only at these very high concentrations were any of the herbicides toxic enough to significantly reduce microbial activity. Furthermore, concentrations greater than 1000 ppm are artificially high levels of herbicide, given that usual field concentrations rarely exceed 100 ppm. Since these herbicides have a low persistence in the forest floor, it is unlikely that any substantial build-up of herbicide could occur. Thus, while it is useful to know toxic thresholds for litter decomposition, it is encouraging that such effects only occur at unrealistically high concentrations.

Forest floor samples treated with 2,4-D exhibited significantly increased decomposition at concentrations greater than or equal to 100 ppm. The phenomenon of 2,4-D stimulation of microbial activity has been observed previously by Bollen (1961), Upchurch (1966), and Atlas (1978).

It should be noted that toxicity of certain herbicides to microbes depends to an extent upon pH of the substrate. At pH less than 6.0, phenoxy herbicide toxicity is reduced, while at pH greater than 6.0, toxicity is increased (Moreale and Van Bladel, 1980). The substrates used in this experiment were ca. pH 4,. so it can be assumed that toxic effects were minimized.

On our spray sites, changes in vegetation, and secondary effects on microclimate, did not exert significant effects on litter decomposition. In parallel vegetation studies using a grid of permanent plots surveyed before and one-year after the spray

treatments (Morash and Freedman, unpublished), a 16% increase was found in total cover of ground vegetation on the unsprayed control plot, but a 14% decrease on the 3.4 kg a.i./ha 2,4,5-T spray plot, and a 23% decrease on the 6.7 kg a.i./ha 2,4,5-T plot. Total shrub stem density and basal area (including Rubus spp.) increased by 30% and 34%, respectively, on the unsprayed plot, while they decreased by 30% and 28%, respectively, on the 3.4 kg a.i./ha 2,4,5-T plot, and by 31% and 28%, respectively, on the 6.7 kg a.i./ha 2,4,5-T plot. However, as noted above, these changes in vegetation and the changes in microclimate they would produce, were not sufficiently large to have measurable effects on litter decomposition among the treatments.

4.4 Conclusions

1) Laboratory studies using three organic substrates indicated that the herbicides 2,4-D; 2,4,5-T; a 50:50 mixture of 2,4-D and 2,4,5-T; and glyphosate all had toxic thresholds for fresh foliage decomposition. However, in all cases the thresholds were much higher than residue concentrations that occur in the field after silvicultural herbicide treatments. It must be acknowledged, however, that microbial populations on fresh leaf litter are not necessarily the same as those in forest floor litter and thresholds obtained in the laboratory studies may not represent field thresholds.

2) A field study at one site found no measurable first-year post-spraying effects on litter decomposition among treatment plots sprayed at 0.0, 3,4, or 6.7 kg 2,4,5-T/ha.

5.0 EFFECTS ON STREAM PERIPHYTON

Pesticides may enter aquatic environments through direct application to surface waters, transport in ephemeral stream channels, overland flow, leaching, drift from nearby spray areas, and wind erosion (Norris, 1981; NRCC, 1978).

Glyphosate is a herbicide used in conifer release programs and, as a consequence, may be broadcast near watercourses. It is highly water soluble and, in a distilled aqueous solution at room temperatures, is stable for many years (Bronstad and Friestad, 1985). The chemical is degraded by microbes, however, with aminomethyl phosphonic acid being the major metabolite (Rueppel et al., 1977). A number of other mechanisms including adsorption to available surfaces, and dilution, may account for the rapid disappearance of glyphosate from impacted natural water bodies (estimated half-life 12 h) (Tooby, 1985).

The short term toxicity of glyphosate and its commercial formulation, Roundup ^{R}, to several species of aquatic organisms has been established. Folmar et al. (1979) determined that the 96 h LC50 of Roundup ^{R} for rainbow trout, fathead minnows, channel catfish, bluegills and scuds were 8.3, 2.3, 13.0, 5.0 and 43 mg/L respectively. These authors also observed, however, that rainbow trout fingerlings were much more sensitive to Roundup ^{R} (96 h LC50; 1.3 mg/L) than adult fish and that the formulated product Roundup ^{R} was slightly more toxic than the active ingredient, glyphosate.

Changes in primary production may also be an important consideration with herbicide contamination of watercourses (NRCC, 1978). There is little information available on the effect of glyphosate on microflora. Sullivan et al. (1981) were unable to relate changes in abundance of diatoms of a stream and pond to treatment with Roundup ^{R} at a rate of 2.2 kg a.i./ha.

The purpose of the present study was to determine aquatic residue concentration following forestry application of Roundup ® and, in addition, to ascertain if such concentrations can affect stream periphyton abundance.

5.1 Materials and Methods

A small stream, Bryden Brook, in Pictou County, Nova Scotia, was selected within an operational conifer release spray block of Nova Scotia Forest Industries (Figure 3). This stream drained an area that had been burned over 10 years previously. The stream had a mean flow rate of approximately 1700 L/min. Water quality characteristics of the stream water are contained in Table 8. The stream had a gravel bottom and a closed canopy of willow (Salix spp.), alder (Alnus spp.) and black spruce (Picea mariana).

One month prior to spray, 72 multiplate artificial substrate samplers (12 at 6 sites) were placed in the stream above (control) and below (treatment) the area to be treated. Samplers consisted of 10 pieces of masonite 4 x 75 x 75 mm bolted through their centres and separated by 4 mm spacers. The masonite used was rough on one side and smooth on the other. These samplers were placed on the stream bottom, in riffles. Samplers were tethered by short lines to steel rods driven into the streambed.

On September 12th, the stream was treated with two overlapping swaths of Roundup ® herbicide delivered at a rate of 2.0 kg a.i./ha in 55 L water from a Bell 206 Jet Ranger helicopter equipped with boom and nozzles (Tee Jet D6 cones) and flying approximately 20 metres above the ground parallel to the stream. The herbicide was applied to a point approximately 200 metres upstream of the first treatment area sampler.

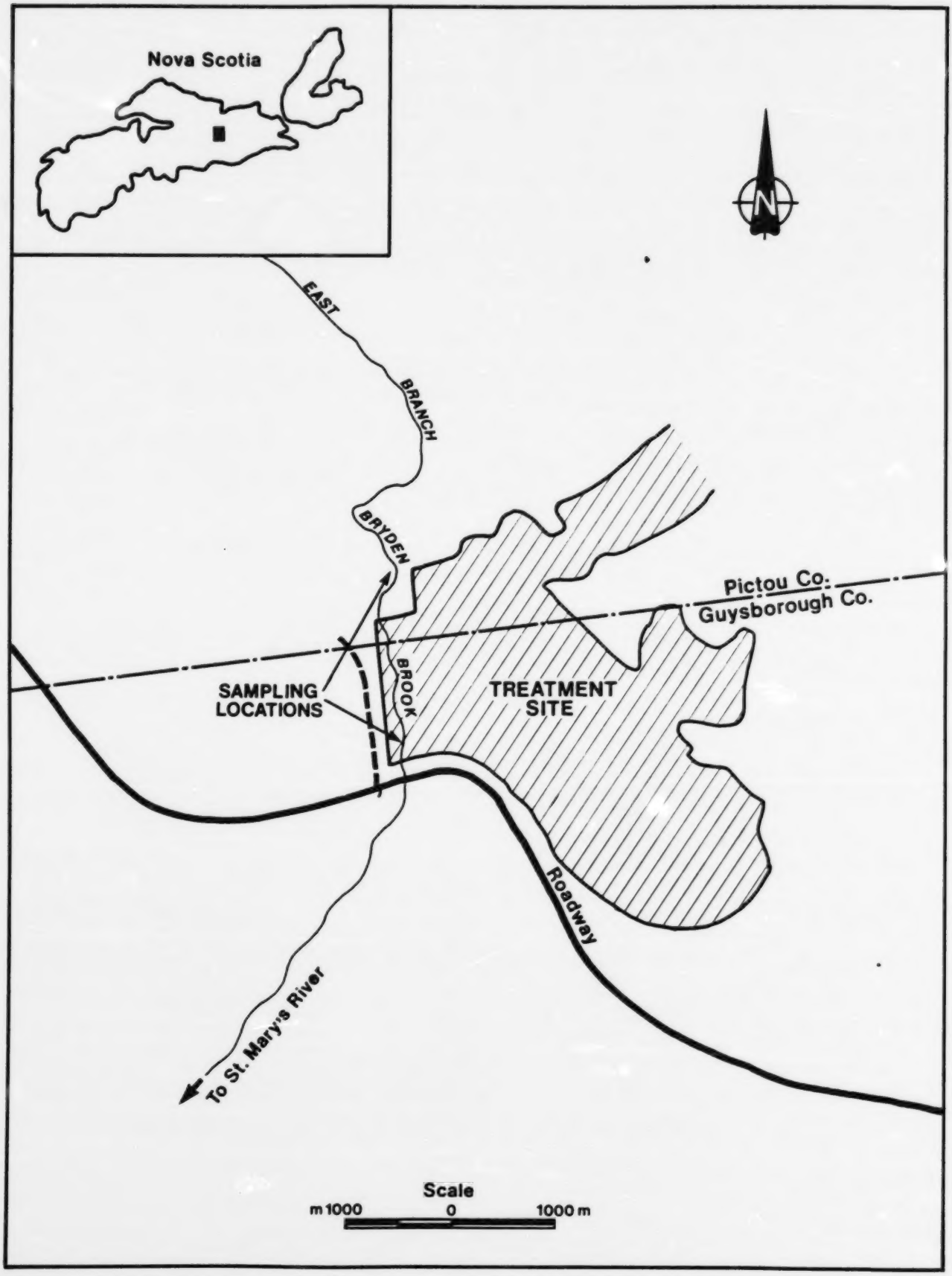


FIGURE 3 - BRYDEN BROOK SAMPLING LOCATION PICTOU CO. N.S.

TABLE 8 WATER QUALITY CHARACTERISTICS OF BRYDEN BROOK (mg/L)

Variable	Mean Concentration (± 1 S.D.)	Range	No. of Observations
Hardness	3.5 ± 0.5	-	6
Alkalinity	L 2.0	-	6
NO_3	L 0.02	-	6
NO_2	L 0.02	-	6
Total Phosphorus	-	L 0.005 - 0.18	6
Total Dissolved Solids	36.0 ± 4.2	-	5
Total Dissolved Carbon	7.0 ± 1.1	-	6
pH	-	5.1 - 5.4	6
Temperature (°C)	-	11 - 17	4

L = less than

At various intervals prior to, and after spray, 4-6 samplers were removed from the treated and control portions of the stream. Samplers were immediately disassembled and 8 of the 10 plates sealed separately in foil food trays, placed on dry ice and transported to the EPS laboratory where they were stored at -20°C until analyzed for chlorophyll and total biomass content. The surfaces of the two remaining plates from each sampler were scraped, both sides, with a glass microscope slide and the scrapings placed immediately into a glass tube containing 3 mL of boiling Tris buffer. The sides of the tube were then rinsed with an additional 2 mL of boiling Tris buffer, dispensed with a disposable pipet. The tube was placed in a boiling water bath for 5 min., allowed to cool and then placed on dry ice. Samples were stored at -20°C until analyzed for ATP content (APHA, 1975).

Two plates from each sampler were analyzed for chlorophyll/phaeophytin. They were thawed and the material on both sides scraped into an 85% acetone/water solution. The fluoresence of the resultant solution was measured using a Turner Design Model 10 fluorometer with appropriate settings to maximize detection. After recording a response, 200 uL of 0.2 N hydrochloric acid was added and the fluoresence response again measured. The two values, before acidification (R_B) and after acidification (R_A), were then used to calculate the chlorophyll *a* and phaeophytin concentrations per masonite board using the following formulae:

$$\text{chorophyll } \underline{a} \text{ (mg)} = F_D \left(\quad \right)^{-1} (R_B - R_A)$$

$$\text{phaeophytin (mg)} = F_D \left(\quad \right)^{-1} (R_A - R_B)$$

where = mean of all R_B/R_A ratios during calibration and

F_D = calibration constant.

All analyses were conducted in duplicate. In addition the chlorophyll/phaeophytin ratio was calculated.

Two plates from each sample were analyzed for total dry biomass. Biological material was scraped into weigh boats and dried to a constant weight at 105°C.

The samples to be analyzed for ATP content were throughly mixed using a Vorlex mixer and then 0.2 mL of enzyme (firefly lantern extract - Sigma Chemical Co. Ltd.) was combined with 0.2 mL of sample or standard. Reference standards, with a range of 1×10^{-2} ug ATP/200 uL to 1×10^{-4} ug ATP/200 uL, were prepared from an ATP standard and sterile stock Tris buffer solution. Standards were analyzed before and after each set of samples. Values of ug ATP/200 uL were calculated using the equation:

Sample CPM (counts per minute) = (enzyme + sample CPM) - (enzyme CPM).

The total for the 2 plate composite samples were calculated from this. Sample luminescence was determined on a JRB Inc. Model 2000 ATP photometer.

Prior to, and at increasing time intervals after spray, surface water samples (3 L) were taken in amber glass bottles (3 x 1L) which had been previously detergent washed, rinsed with distilled water and then with acetone and pesticide grade hexanes. Samples were frozen and stored at -20°C until analyzed for glyphosate and aminomethyl phosphoric acid (AMPA) content according to the method outlined in the FDA Pesticide Analytical Manual (Anon, 1982).

5.2 Results

The results of analyses for glyphosate and AMPA in streamwater are contained in Table 9. Concentrations of parent glyphosate were variable, with the highest concentration being recorded 30 hours after spray. Measurable quantities of glyphosate were detected in the pre-spray sample. The metabolite AMPA was

positively identified in only four samples, with the greatest concentration noted at 30 hours after spray. No rain occurred during the sampling period.

Table 10 contains the results of chlorophyll a, phaeophytin, total biomass and ATP analyses from artificial substrates. Chlorophyll a content of plates from treated portions of the stream were generally lower than those taken from the control portion of the stream, with the exception of the 6 day post-spray samples.

The chlorophyll/phaeophytin ratio did not change substantially in the treated or control portion of the stream during the measurement period. Although it was somewhat greater in the treated portion of the stream, the difference was not significant (t-test, $p < 0.05$).

The measurements of total biomass were highly variable. There appeared to be a decline in biomass in the treated portion of the stream after spray, however, this was accompanied by a similar decline in the control portion of the stream. The data may have been confounded by changes in rates of colonization and possible die-back since the experiment was done during the autumn.

The ATP values reported were very close to the analytical detection limit. Results were again variable and no clear trends in the data were observable.

5.3 Discussion

It has been demonstrated (Goldsborough and Robinson, 1983; Kosinski, 1984) that herbicides can have effects on periphyton. Since periphyton communities represent almost the sole opportunity for accumulation and retention of dissolved nutrients in lotic ecosystems (Rodgers et al., 1979), impacts at this level could have serious

TABLE 9 CONCENTRATION OF GLYPHOSATE AND AMINO METHYL PHOSPHONIC ACID (AMPA) IN STREAMWATER

Time	Chemical Concentration (ug/L)	
	Glyphosate	AMPA
Pre-spray	0.33	L 0.1
1 h Post-spray	7.6	L 0.1
2 h Post-spray	1.2	L 0.1
5 h Post-spray	0.93	0.14
12 h Post-spray	0.79	0.28
15 h Post-spray	18.0	0.10
24 h Post-spray	0.18	L .10
30 h Post-spray	39.0	0.55

TABLE 10 PERIPHYTON ABUNDANCE ON ARTIFICIAL SUBSTRATES.[a]

Time	Chlorophyll a (mg)	Phaeophytin (mg)	Chorophyll/ Phaeophytin	Total Biomass (dry wt.) (mg)	ATP (ug)
Treated Site					
2 days Pre-spray	4.3 ± 2.5(10)	7.6 ± 3.1 (10)	0.6 ± 0.3 (10)	349.4 ± 271.9(5)	N.D.[b](10)
1 day Post-spray	4.2 1.1(10)	5.9 ± 1.4 (10)	0.7 ± 0.2 (10)	189.4 ± 85.9(5)	9.41 + 10.85(8) N.D.(2)
6 days Post-spray	29.4 ± 29.9(10)	32.8 ± 29.3 (10)	0.8 ± 0.2 (10)	157.6 ± 134.6(5)	3.51 N.D.(9)
20 days Post-spray	5.9 ± 2.6(10)	10.3 ± 3.5 (10)	0.6 ± 0.2 (10)	346.6 ± 187.4(5)	10.27 + 6.74(6) N.D.(4)
37 days Post-spray	4.0 ± 2.3(8)	8.7 ± 4.2 (8)	0.4 ± 0.1 (8)	441.5 ± 349.5(4)	3.10 + 0.23(4) N.D.(4)
Control Site					
2 days Pre-spray	6.5 ± 1.7(4)	12.7 ± 3.5 (4)	0.5 ± 0.05(4)	821 (2) 763 - 879	N.D.(4)
1 day Post-spray	5.4 ± 2.0(6)	7.6 ± 2.1 (4)	0.7 ± 0.2 (6)	494.3 (3) 72 -1060	2.17 ± 0.67(6)
6 days Post-spray	5.4 ± 2.4(6)	10.1 ± 3.0 (6)	0.5 ± 0.1 (6)	629.3 (3) 375 -1312	1.66 - 1.74 N.D.(4)
20 days Post-spray	18.4 ± 17.6(5)	73.2 ±105.23(6)	0.5 ± 0.3 (6)	402 (3) 52 - 683	12.95 - 12.30 N.D.(4)
37 days Post-spray	11.9 ± 21.3(8)	17.1 ± 23.5 (8)	0.5 ± 0.2 (8)	209.5 ± 99.4(4)	9.24 + 6.45(4) N.D.(4)

[a] Values stated are means ± 1 standard deviation. Numbers in brackets are total number of analyses.
[b] Expressed as combined amount on 2 masonite plates, total area 225 cm^2.
N.D. indicates not detected.

consequences. The fact that this study did not identify any significant effects attributable to the glyphosate treatments is, therefore, a significant finding.

Several factors may have contributed to this lack of effects.

The first of these is that glyphosate residues in the stream water were very low, considering the overspray situation. Since the stream bed had an average depth of 15 cm, the emitted rate of 2.0 kg a.i./ha should have resulted in a water column concentration of 1.3 mg/L. This calculated maximum concentration is more than 33 times greater than the maximum observed residue concentration of 0.039 mg/L. The calculation, however, assumes even and total deposit of emitted pesticide, rapid mixing within the water column, and no "attenuating" forces acting on the pesticide concentration in the water column.

As noted earlier (page 11 and 12), assumptions of uniform, high deposit within swaths are unrealistic. In this case, the closed canopy of riparian vegetation would also have intercepted a portion of the emitted spray. Uneven distribution of pesticide within the water column is probably not a major factor since Eidt et al. (1984) have shown that pesticides are rapidly mixed in small, fast flowing streams. Attenuating factors such as adsorption to sediments (Tooby, 1985) and absorption by aquatic macrophytes (Eidt and Weaver, 1984) cannot be discounted. It has been observed, however, that 79-91% of the Roundup ^{R} applied directly to a stream could be accounted for at a sampling point 0.3 km downstream from the point of application, 70% at a point 1.6 km downstream, and 58% 8 km downstream (Comes et al., 1976). This would seem to indicate that, in stream situations at least, Roundup ^{R} does not disappear rapidly from the water into which it is introduced. This strengthens the argument that in this study a large proportion of the herbicide must not have reached the stream water surface, probably due to the closed canopy above the stream.

Other observers have noted effects on periphyton when exposed to simazine, terbutryn (Goldsborough and Robinson, 1983), atrazine and paraquat (Kosinski, 1984) at the 0.01 to 0.1 mg/L level. Glyphosate appears to be relatively less toxic to periphyton since the residues measured in this study were within the above range and we noted no significant periphyton impacts were noted. The variability of the data precluded the possibility of determining whether small changes in periphyton abundance may have occurred.

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